REMARKS

With respect to the §§ 102 and 103 rejections of claims 29-39, 41, 46-52 and 59-61, the primary basis for those rejections is the cited Chen et al. patent (hereinafter "Chen patent"). As discussed in greater detail below, the Examiner appears to be comparing alleged similarities between the Chen patent and applicant's claimed invention, while overlooking significant differences between the claimed invention and the Chen patent. Briefly, the present application discloses two methods for isolation of RNA. One claimed method utilizes phase separation and the other utilizes acidic phenol precipitation to selectively precipitate DNA, proteins and other molecules while leaving RNA in a soluble form. Both of these methods are significantly different than the method described in the Chen patent.

Chen method.

The method for extracting RNA described in the Chen patent is based on phase separation and subsequent sequestering of RNA into an aqueous phase under conditions specified in claims 1 and 6 of Chen.

Composition of reagents and the method in the Chen patent were an attempt to improve my previous invention (see page 5, one-step method and TRIzol reagent), published in Anal. Biochem. **162**, 156, 1987 and US Patents Nos. 4,843,155 and 5,346,994, granted in 1989 and 1994, respectively. The Chen patent modifies the method of my previous invention by substantially reducing buffering salt concentration, adding detergents and extending the pH range from pH 4 - 6 to pH 3.5 - 6.5. In addition, specific limits are claimed on concentrations of the used components. No rationale is provided for each of the introduced changes and limits.

The Chen patent is evaluated in comparison to the TRIzol reagent product. The Chen patent discloses that the quality of the extracted RNA is slightly better than that of TRIzol (page 10, last sentence), even though no data have been provided to support this statement. It has been confirmed that only a limited improvement, if any, over the single-step method was made by the Chen patent by showing that RNA isolated as described in the Chen patent is significantly contaminated with DNA (Piotr Chomczynski declaration of January 17, 2007). This contamination was detected using a 30-cycle PCR followed by the Northern blotting.

In addition, purity of the RNA isolated in the Chen patent, as regards protein contamination, is questionable. In accord with the Inventor statement (claim 6.10), it is required to dissolve the isolated RNA in the "RNA protectant" solution. The use of "RNA protectant" to protect the extracted RNA is the requirement stated also in Embodiment 2, p.10, and Embodiment 3, last sentence.

TRIzol reagent, a product of Invitrogen Corporation (Carlsbad, CA) is based on the single-step method performed in accord with applicant's own '155 and '994 patents. Evidence was previously presented that methods of applicant's previous '155 and '994 patents result in isolating RNA contaminated with DNA (current Application, par. 5). Thus, it is not a surprise that the Chen patent only slightly improves the quality of the isolated RNA and does not eliminate DNA contamination.

Differences in Methods.

There are two methods of RNA isolation claimed in the present application.

- Phase separation method.
- Acidic phenol precipitation method.
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The Chen patent issued in 1999 describes the RNA extraction procedure based on phase separation. It is designed to provide "simpler operation and quicker results"(page 5) than that of used in the TRIzol reagent protocol. The TRIzol reagent is a product of Invitrogen Corporation (Carlsbad, CA) a US company formerly named Life Technologies BRL. TRIzol reagent is based on applicant's US patents no. 4,843,155 and 5,346,994 employing phase separation to purify RNA.

The important difference between the methods described in the Chen patent and methods in the present application is the end result, as reflected in the quality of the isolated RNA. Improvement in the purity of the extracted RNA is not the purpose of the Chen invention. The Chen patent was elaborated to simplify manufacturing and for quicker operation. This is stated on page 5 (bottom) and page 6 (top):

"The purpose of this invention is to provide total RNA extraction reagent and manufacturing method that can **shorten the time, simplify manufacturing**, and improve effectiveness of extracting total RNA (including rRNA, tRNA, and mRNA), as well as an RNA extraction method **with simpler operation and quicker results**", emphasis added.

The simplification introduced in the Chen patent results in such a low quality of isolated RNA that at the end of the extraction process its protocol requires the use of an "RNA protectant" to dissolve the extracted RNA. The use of RNA protectant to protect the extracted RNA is the requirement stated in: claim 6.1, Embodiment 2, p.10, and Embodiment 3, last sentence.

This makes it impractical to use RNA extracted by the Chen method in most current applications. Known RNA protectants are, for example, guanidine salts and sodium dodecyl sulphate. These compounds are inhibitors of enzymatic reaction and should be removed before using the RNA solution in tests. Depending on the protectant in use, the Northern blotting might be the only choice for immediate use of RNA isolated in accord with the Chen patent.

RESPONSE TO THE EXAMINER COMMENTS

Detailed Action

Claim Interpretation.

The Examiner refers to the part of the present application containing the new limitation, stating: "a buffer at a concentration sufficient to maintain a pH in the range from about pH 3.6 to below pH 4.0". It is further stated that: "Therefore, any amount of buffer will function to maintain the pH in the desired range when the sample is already in the desired range".

This statement does not reflect the use of the methods of the present invention. The methods of the invention are designed to isolate RNA from biological samples (claims 29, 47, 48, 59 and 61). A biological sample is defined in paragraph 21 and comprises samples from a wide array of sources including samples from humans,

animals and plants. It is well known that most of these samples have pH ranging from pH 7 to pH 8. If the reagent does not have sufficient buffering capacity it could not be used for samples identified in claims 29, 47, 48, 59 and 61.

Claim rejections

RE 2.

No description nor patent was found in the art describing isolation of DNA-free RNA, ready for RT-PCR using either the phase separation method or the acidic phenol precipitation method claimed.

RE 3.

It is noted that the Examiner appears to change the meaning of text in the Chen patent by selectively and inaccurately citing information from the patent.

The Examiner writes on page 2 (bottom): "Chen teaches a method for isolating **purified RNA** from a biological sample of claims 29 and 59. This is not what is written in the Chen patent. The description "**purified RNA**" is never used in the text of Chen patent. The goal (purpose) of the Chen patent is stated on page 5 (bottom) and page 6 (top):

"The purpose of this invention is to provide total RNA extraction reagent and manufacturing method that can **shorten the time, simplify manufacturing**, and improve effectiveness of extracting total RNA (including rRNA, tRNA, and mRNA), as well as an RNA extraction method **with simpler operation and quicker results**", emphasis added.

The Chen patent discloses simplification of the manufacturing method, simpler operation and quicker results. The purity of the extracted RNA is not the purpose of the invention.

RE a.

The Examiner writes "Treating the sample (with a composition) comprising phenol..."

In addition to phenol, the composition for treating the sample in the present application contains other ingredients and conditions that should be used in the claimed methods and that are not present in the Chen patent. For example, in addition to phenol, the present invention includes a buffer in sufficient concentration to maintain pH. Phenol alone is not an inventive ingredient of the claimed invention.

An example is claim 47. In this claim, it is stated that in addition to phenol, a buffer sufficient to maintain pH from about 3.6 to about 5.5 is the only critical ingredient to isolate purified RNA. It should be noted that this claim does not require the presence of guanidine salts. This itself substantially differs this claim from the Chen patent.

RE b.

In part b referring to pH, page 3, the Examiner does not recognize that the Chen patent does not have "buffer at a concentration sufficient to maintain pH in the range from about 3.6 to below 4". 0.005% -0.02 % concentration range of Chen's pH regulator (i.e. buffer) is not sufficient to maintain pH below 4 during the RNA isolation from samples described in the Chen's patent.

On page 8, the Examiner acknowledges that "While Chen teaches the use of a pH adjusting component, Chen does not state that the amount used will be sufficient to maintain pH".

The novelty of the present invention is the use of pH range from about 3.6 to below 4 in isolating RNA free of DNA contamination (as judged by the RT-PCR, par.21). There is no indication in the Chen patent that this pH range is beneficial for the effective removal of DNA. Also, there is no indication in the art of RNA isolation, before the present invention, that performing the phase separation method at pH below 4 is beneficial for DNA removal. To the contrary, it was thought that pH above 4 is more beneficial (see par. 11 of Application).

It should be re-stated that Chen's patent does not address DNA contamination, nor purity of RNA regarding protein contamination. The only comment related to the purity of RNA is the last sentence of the patent text that reads: "The quality of the extracted RNA is slightly better than the imported TRI-zol reagent and the manufacturing cost is lower". The term "slightly better" indicates a similar purity of RNA, though no data are provided in the Chen patent to validate this statement.

The simplification in Chen results in such low quality of the isolated RNA that at the end of the extraction process the protocol in the Chen patent calls for the use "RNA protectant" to dissolve the extracted RNA. The use of RNA protectant to protect the extracted RNA is the requirement stated in: claim 6.1, Embodiment 2, p.10, and Embodiment 3, last sentence. This disqualifies the use of RNA extracted by the Chen method in most applications. Known RNA protectants are, for example, guanidine salts and sodium dodecyl sulphate. These compounds are known inhibitors of enzymatic

activity.

Claim 59 describes selective precipitation of higher molecular weight RNA. This method allows isolation of the high and low molecular weight RNA as separate fractions. There is no comparable method for isolating RNA in the Chen patent. The precipitation method with one volume of isopropanol precipitates, used in the Chen patent, precipitates both, low and high molecular weight RNA, not separately.

Claim 29 describes a method for isolating purified RNA. The purified RNA is free of DNA contamination when assayed by RT-PCR (Application, par. 21). There is no claim in the Chen patent that isolated RNA is free of DNA.

RE claims 47-49 (page 4).

There is a major difference in the method of acidic phenol precipitation of claims 47- 49. The pH requirement for the acidic phenol precipitation method is 3.6 to 5.5 and differs from the pH range of the phase separation method.

There is no chloroform-induced phase separation in the acidic phenol precipitation method. The chloroform-induced phase separation is a required, and critical, part of the method presented in Chen patent. Thus, the difference between these two methods comprise both the reagents and purification steps involved in both methods. The elimination of phase separation described in the present invention is advantageous and allows for substantial simplification of the RNA isolation (see par. 35-38).

There is no indication in the Chen patent that chloroform-induced phase separation can be eliminated from the protocol. It is the integral part pf Chen patent: claim 6.2; page 8, Embodiment 2, p.2; page 9, Embodiment 3, chloroform use "to extract the separated RNA".

The pH requirement of the acidic phenol

At the bottom of page 4, the Examiner refers to use of sedimentation" that will remove DNA". First, there is no remark in the Chen patent that DNA was removed during the isolation process. As mentioned, DNA is **not** effectively removed in the Chen patent. Second, sedimentation is not part of the present invention nor in the Chen patent. The inventive part of the claimed method is to create conditions to selectively precipitate DNA and proteins and then to separate these components from RNA by sedimentation.

RE claim 50 (Examiners comments, page 5)

Claim 50 is an adjunct claim to the Inventive claims 47 and 48.

RE 51.

There is no statement in the Chen patent regarding any composition which can be at "about 1.5 X" concentration Neither on page 8, as Examiner indicates, nor in the rest of the Chen patent text.

Regarding "prima facie obvious" comments on page 8.

The present invention is about providing RNA that is substantially free of DNA and can be used in the RT-PCR without additional purification. To date, no practitioner has indicated nor suggested that it is achievable by a simple modification of the existing phase separation method. Historical evidence indicates that since 1999

when Chen patent was published no practitioner has made efforts to change the Chen method the way the Examiner suggests. The trend to improve the single-step method was, contrary to the present invention, to increase pH to above 4. It is not that practitioners did not try to improve performance of the phase separation method. The most widely used existing improvements combined a phase separation method with silica column methods.

Comments on page 11 - Unpredictability

The Examiner erroneously assumes that the declaration of 17 January indicates that pH 3.5 did not function. In the example used in the January 17, 2007 declaration a reagent was used that was prepared according the Chen patent. The insufficient buffering results when the conditions claimed in Chen patent are used, and the pH of a sample homogenate is above pH 4. The importance of buffering capacity and pH range is discussed in previous section of this response.

What was shown in the January 17 declaration was that a reagent prepared in accord with the present invention provides DNA-free RNA, while a reagent prepared in accord with the Chen patent does not work properly in this respect.

The defective outcome of the Chen patent is clearly due to differences in the reagent compositions. The contributing factors can be: pH above 4, too low a concentration of buffering salt, and too high a detergent concentration, as compared with reagents for phase separation disclosed in the present application. .

Another error in the Examiner comments is to associate effectiveness of pH 4.2 with the phase separation method. That is not what is claimed in the present application. There are **two methods** claimed: one phase separation method with pH

range from about 3.6 to below 4. The second is the acidic precipitation method with the pH range from about 3.6 to about 5.5. Thus, all references of the Examiner to the use of pH 3.5 and unpredictability of methods of the present invention are moot.

CONCLUSION

In view of the foregoing remarks, Applicant believes this case is in condition for allowance and respectfully requests allowance of the pending claims. If the Examiner believes any issue requires further discussion, the Examiner is respectfully asked to telephone the undersigned attorney so that the matter may be promptly resolved. The Examiner's prompt attention to this matter is appreciated.

Applicants do not believe that any fees are due in connection with this submission. However, if such petition is due or any fees are necessary, the Commissioner may consider this to be a request for such and charge any necessary fees.

Respectfully submitted,

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